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May 29, 2014

TSCA Confidential Business Information Center (7407M)
EPA East – Room 6428
US Environmental Protection Agency
1201 Constitution Avenue, NW
Washington, DC 20004-3302
Attention: TSCA Section 8(e) Coordinator

Results of an *in vitro* mammalian cell gene mutation test (HPRT-locus) in Chinese Hamster V79 cells with ARALDITE PT 910 (TK 30041).

The European office of the Huntsman Advanced Materials Division (Huntsman) has received a draft final report from an *in vitro* HPRT gene mutation assay using ARALDITE 910 (TK 30041). This study was conducted using a standard OECD 476 protocol by BSL BIOSERVICE in Germany. The results of this genetic toxicity study indicate that this product is considered to be mutagenic in this test system. While this information has been obtained from a "draft final" report, the final report is not expected to be substantially different from this report with regard to the conclusions of the study.

Huntsman is submitting this information pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA). Huntsman has not made a determination as to whether a significant risk of injury to human health or the environment is actually presented by these findings.

The results of the study can be summarized as follows:

- The HPRT gene mutation assay protocol used in this mutagenicity evaluation of the test substance employed two independent experiments. Experiment I consisted of a short term (4 hour) treatment period with and without S9 metabolic activation. Experiment II consisted of a short term (4 hour) treatment period with S9 metabolic activation (confirmation of results of Experiment I, and a long term (20 hour) treatment period without S9 activation).
- No precipitate of the test substance was observed at any tested concentration.
- No increase of mutants was observed following treatment with the test substance in Experiment I (4 hour treatment without S9). A dose-response relationship was not observed.
- The test substance induced biologically significant increases in mutant frequency in Experiment I (4 hour treatment with S9), and in Experiment II (4 hour treatment with S9 and 20 hour treatment without S9).
- Positive and negative controls were within historical limits for the laboratory.
- The study director concluded that, under the experimental conditions reported, the test substance is considered to be mutagenic in the *in vitro* HPRT gene mutation assay using Chinese Hamster V79 Cells.

A copy of the draft final report is attached.

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As always, if I can provide any additional information on the above study, please call me at (281) 719-3017, or contact me via e-mail at: Ray_Papciak@Huntsman.com.

Regards,

Raymond J. Papciak
Manager, Product Safety

In vitro Mammalian Cell Gene Mutation Test (HPRT-Locus) in Chinese Hamster V79 Cells with Araldite PT 910 (TK 30041)

Report

Version: Draft 01 / Date: 14 May 2014

Study Completion Date: .....

BSL BIOSERVICE Study No.: 137232

Sponsor:
Huntsman Advanced Materials Europe (BVBA)
Everslaan 45
B-3078 Everberg
Belgium

1. Copy of the GLP Certificate

A copy of the GLP certificate will be included in the final report.

2. Contents

3. List of Tables

4. Preface

4.1. Abbreviations

Art.	Artikel *(article)*
BGBl.	Bundesgesetzblatt *(Federal Law Gazette)*
CE	cloning efficiency
DMBA	7,12 dimethylbenzanthracene
DMSO	dimethylsulfoxide
e.g.	exempli gratia *(for example)*
EC	European Community
EMS	ethylmethanesulfonate
EPA	Environmental Protection Agency
FBS	fetal bovine serum
GLP	Good Laboratory Practice
GmbH	Gesellschaft mit beschränkter Haftung *(company with limited liability)*
HPRT	hypoxanthine-guanine-phosphoribosyl-transferase
MEM	minimal essential medium
No	number
Nr.	Nummer *(number)*
OECD	Organisation for Economic Cooperation and Development
PBS	phosphate buffered saline
QA	Quality Assurance
QAU	Quality Assurance Unit
SC	survived cells
SOPs	Standard Operating Procedures
TG	thioguanine
v/v	volume per volume

4.2. General

Sponsor:	Huntsman Advanced Materials Europe (BVBA) Everslaan 45 B-3078 Everberg Belgium
Study Monitor:	Mr Matthieu Ott Huntsman Advanced Materials Switzerland K402.4.06 Klybeckstrasse 200 CH-4057 Basel Switzerland
Test Facility:	BSL BIOSERVICE Scientific Laboratories GmbH Behringstraße 6/8 82152 Planegg Germany
BSL BIOSERVICE Study No.:	137232
Test Item:	Araldite PT 910 (TK 30041)
Title:	*In vitro* Mammalian Cell Gene Mutation Test (HPRT-Locus) in Chinese Hamster V79 Cells with Araldite PT 910 (TK 30041)

4.3. Project Staff

Study Director:	Dr. Barbara Wallner
Management:	Dr. Wolfram Riedel Dr. Angela Lutterbach Dr. Katrin Witschital
Head of GLP Quality Assurance Unit:	Dipl.-Biol. Uwe Hamann

4.4. Schedule

Arrival of the Test Item:	03 December 2013
Study Initiation Date:	15 January 2014
Experimental Starting Date:	15 January 2014
Experimental Completion Date:	08 May 2014

5. Quality Assurance

5.1. GLP Compliance

This study was conducted to comply with:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on August 28, 2013 (BGBl. I S. 3498) [1].

Konsens-Dokument der Bund-Länder-Arbeitsgruppe Gute Laborpraxis ("Consensus Document of the National and Länder Working Party on Good Laboratory Practice") on the archiving and storage of records and materials, 5 May 1998 [2].

OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998 [3].

This study was assessed for compliance with the study plan and the Standard Operating Procedures of BSL BIOSERVICE. The study and/or the test facility are inspected periodically by the Quality Assurance Unit according to the corresponding SOPs. These inspections and audits are carried out by the Quality Assurance Unit, personnel independent of staff involved in the study. A signed quality assurance statement, listing all performed audits, is included in the report.

5.2. Guidelines

This study followed the procedures indicated by internal BSL BIOSERVICE SOPs and the following internationally accepted guidelines and recommendations:

Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 476, "*In vitro* Mammalian Cell Gene Mutation Tests" adopted July 21, 1997 [4].

Commission Regulation (EC) No 440/2008, L142, Annex Part B, B 17, "*In vitro* Mammalian Cell Gene Mutation Test", dated May 30, 2008 [5].

EPA Health Effects Test Guidelines, OPPTS 870.5300 "*In vitro* Mammalian Cell Gene Mutation Test" EPA 712-C-98-221, August 1998 [6].

5.3. Archiving

The records, materials and specimen will be stored according to the GLP regulations for a period of 15 years.

The following records have to be stored according to the GLP regulations:

A copy of the final report, the study plan and a documentation of all raw data generated during the conduct of the study (documentation forms as well as any other notes of raw data, printouts of instruments and computers) and the correspondence with the sponsor concerning the study. Any document relating to the study will be discarded only with the prior consent of the sponsor.

The following materials and samples have to be stored according to the period of time specified in the GLP regulations:

A retain sample of the test item will be archived according to the GLP regulations, if possible, and will be discarded without the sponsor's prior consent.

Other materials and specimen have to be stored according to the GLP regulations and disposed after the respective archiving period with the sponsor's prior consent.

As requested the remaining test item will be returned to the sponsor.

6. Statement of Compliance

BSL BIOSERVICE Study No.:	137232
Test Item:	Araldite PT 910 (TK 30041)
Title:	*In vitro* Mammalian Cell Gene Mutation Test (HPRT-Locus) in Chinese Hamster V79 Cells with Araldite PT 910 (TK 30041)
Study Director:	Dr. Barbara Wallner

This study performed in the test facility BSL BIOSERVICE Scientific Laboratories GmbH was conducted in compliance with Good Laboratory Practice Regulations:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on August 28, 2013 [1].

Konsens-Dokument der Bund-Länder-Arbeitsgruppe Gute Laborpraxis ("Consensus Document of the National and Länder Working Party on Good Laboratory Practice") on the archiving and storage of records and materials, 5 May 1998 [2].

"OECD Principles of Good Laboratory Practice (as revised in 1997)", Paris 1998 [3].

There were no circumstances that may have affected the quality or integrity of the study.

Study Director: Dr. Barbara Wallner

.....

Date: .....

This statement does not include the solubility test.

7. Statement of the Quality Assurance Unit

BSL BIOSERVICE

Study No.:	137232
Test Item:	Araldite PT 910 (TK 30041)
Title:	*In vitro* Mammalian Cell Gene Mutation Test (HPRT-Locus) in Chinese Hamster V79 Cells with Araldite PT 910 (TK 30041)
Study Director:	Dr. Barbara Wallner

This report and the conduct of this study were inspected by the Quality Assurance Unit on the following dates:

Phase of QAU Inspection	Date of QAU Inspection	Date of Reporting to the Study Director and Management
Audit Final Study Plan:	15 January 2014	15 January 2014
Audit Experimental Phase (study-based):	22 January 2014	22 January 2014
Audit Final Report:		

This report reflects the raw data.

Member of the
Quality Assurance Unit:

.....
Print Name:

Date: .....

This statement does not include the solubility test.

8. Summary

8.1. Summary Results

The test item Araldite PT 910 (TK 30041) was assessed for its potential to induce mutations at the HPRT locus using V79 cells of the Chinese Hamster.

The selection of the concentrations was based on data from the pre-experiments. Experiment I **with** and **without** metabolic activation and experiment II **with** metabolic activation were performed as a 4 h short-term exposure assay. Experiment II **without** metabolic activation was performed as 20 h long time exposure assay.

The test item was investigated at the following concentrations:

Experiment I

without metabolic activation:

0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5 and 10 µM

and **with** metabolic activation:

2.5, 5, 10, 25, 50, 75, 100, 200, 300 and 400 µM

Experiment II

without metabolic activation:

0.05, 0.10, 0.25, 0.50, 0.75, 1, 2, 4, 6 and 8 µM

and **with** metabolic activation:

60, 130, 160, 190, 220, 250, 280 and 310 µM

No precipitation of the test item was noted in the experiments.

Biologically relevant growth inhibition was observed in experiment I and II **with** and **without** metabolic activation. In experiment I **without** metabolic activation the relative growth was 10.4% for the highest concentration (10 µM) evaluated. The highest biologically relevant concentration evaluated **with** metabolic activation was 400 µM with a relative growth of 11.4%. In experiment II **without** metabolic activation the relative growth was 18.6% for the highest concentration (8 µM) evaluated. The highest concentration evaluated **with** metabolic activation was 310 µM with a relative growth of 18.1%.

No biologically relevant increase of mutants was found after treatment with the test item in experiment I (**without** metabolic activation). No dose-response relationship was observed.

Biologically relevant increase of mutants was found after treatment with the test item in experiment I (**with** metabolic activation) and in experiment II (**with** and **without** metabolic activation). Moreover, a dose-response relationship was observed in experiment I (**with** metabolic activation).

DMBA and EMS were used as positive controls and showed distinct and biologically relevant effects in mutation frequency.

8.2. Conclusion

In conclusion, in the described mutagenicity test under the experimental conditions reported, the test item Araldite PT 910 (TK 30041) is considered to be mutagenic in the HPRT locus using V79 cells of the Chinese Hamster.

9. Introduction

9.1. Aim of the Study

Mammalian cell culture systems are used to detect mutations induced by chemical substances. This *in vitro* experiment was performed to assess the potential of the test item to induce gene mutations by means of a HPRT (hypoxanthine-guanine-phosphoribosyl-transferase) assay using the Chinese Hamster V79 cell line. The HPRT system detects base pair mutations, frameshift mutations and small deletions [7]. These are considered to be an initial step in the process leading to carcinogenesis. These cells are exposed to different concentrations of the test item, both with and without metabolic activation for a suitable period of time and subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection [7], [10], [11].

HPRT catalyses the conversion of the non-toxic 6-TG (6-thioguanine) to its toxic phosphorylated derivative. Cells deficient in HPRT are selected by resistance to 6-thioguanine. The deficiency of the "salvage" enzyme HPRT means that antimetabolites such as 6-TG are not incorporated into cellular nucleotids and the nucleotids needed for cellular metabolism are obtained solely from de novo synthesis. However, in the presence of the HPRT-enzyme, 6-TG is incorporated into cellular nucleotides, resulting in inhibition of cellular metabolism and cytotoxicity. Thus mutant cells are able to proliferate in the presence of 6-TG, whereas normal cells, which contain HPRT, are not [9]. Cells as monolayer cultures are exposed to the test item for a defined period of time (4 h for short time exposure or 20 h for long time exposure). Cytotoxicity is determined by measuring the growth rate of the cultures.

The treated cultures are maintained in growth medium for 5-8 days to allow near-optimal phenotypic expression of induced mutations. Mutant frequency is determined by seeding defined numbers of cells in medium containing the selective agent (6-TG) to detect mutant cells and in medium without selective medium to determine the cloning efficiency. After a suitable incubation time, cell colonies are counted. The number of mutant colonies in selective medium is adjusted by the number of colonies in non-selective medium to derive the mutant frequency [9].

There is no requirement for the verification of positive results. Negative or equivocal results should be clarified by further testing using modified experimental conditions. Study parameters which might be changed are concentrations, treatment time or metabolic activation conditions.

To establish a concentration response of the test item at least eight analysable concentrations (single cultures) are tested. These concentration levels should yield a concentration related toxic effect. The highest concentration should induce a reduced level of survival of approximately 10-20% relative survival. The lowest concentration should be in the range of the negative control with respect to cell viability and proliferation.

For soluble, non-toxic test compounds the recommended maximum test concentration will be 5 mg/mL, 5 µL/mL or 10 mM, whichever is the lowest. Solvent or negative controls will be tested in duplicate.

Reference mutagens are tested in parallel to the test item in order to demonstrate the sensitivity of the test system.

9.2. Justification for the Selection of the Test System

The OECD Guideline for Testing of Chemicals Section 4, No 476 – *In Vitro* Mammalian Cell Gene Mutation Test – recommends using V79 cell lines of the Chinese Hamster.

10. Materials and Methods

10.1. Characterisation of the Test Item

The identity of the test item was inspected upon delivery at the test facility (e.g. test item name, batch no. and additional data were compared with the label) based on the following specifications provided by the sponsor.

Name:	Araldite PT 910 (TK 30041)
Chemical Name:	Reaction mass of bis(2,3-epoxypropyl) terephthalate and tris(oxiranylmethyl) benzene-1,2,4-tricarboxylate
Common Name:	TK 30041
Batch No.:	AAC0647400
Physical State:	solid/granulate
Storage Conditions:	at room temperature
Molecular Weight (Base Form):	278 g/mol
Purity:	100%
Date of Analysis:	07.11.2013
Expiry Date:	17.05.2015
Safety Precautions:	The routine hygienic procedures were sufficient to assure personnel health and safety.

10.2. Preparation of the Test Item

The test item was dissolved in DMSO,diluted prior to treatment and added directly to the cells in medium (MEM). The solvent was compatible with the survival of the cells and the S9 activity. The pH-value detected with the test item was within the physiological range. Osmolality of 5 mM test item concentration was 460 mOsm/kg (solvent control: 450 mOsm/kg).

10.3. Controls

Negative and solvent as well as positive controls were included in each experiment.

Negative/Solvent Controls

Negative controls (treatment medium, duplicate cultures) and solvent controls (DMSO, Applichem Lot No. 3T003218) were treated in the same way as all dose groups. Since DMSO was used as solvent, extra solvent controls (1% solvent v/v) were added.

Positive Controls

Without metabolic activation

Name:	EMS; ethylmethanesulfonate
Supplier:	Sigma
Catalogue No.	M 0880
Batch No.	BCBK5968V
Dissolved in:	Medium (MEM)
Final concentration:	300 µg/mL

With metabolic activation

Name:	DMBA; 7,12-dimethylbenz(*a*)anthracene
Supplier:	Sigma
Catalogue No.:	D 3254
Batch No.	SLBF3276V
Dissolved in:	DMSO, dimethylsulfoxide; final concentration in medium (MEM) 1%
Final concentrations:	0.8 and 1.0 µg/mL

The dilutions of the stock solutions of the positive controls were prepared on the day of the experiment and used immediately.

The stability of both positive control substances in solution is proven by the mutagenic response in the expected range.

10.4. Test System

10.4.1. Cells

V79 cells *in vitro* have been widely used to examine the ability of chemicals to induce cytogenetic changes and thus identify potential carcinogens or mutagens. These cells are characterized by their high proliferation rate (12 - 14 h doubling time of the BSL BIOSERVICE stock cultures) and their high cloning efficiency of untreated cells, usually more than 50%. These facts are necessary for the appropriate performance of the study.

The V79 cells (ATCC, CCL-93) were stored over liquid nitrogen (vapour phase) in the cell bank of BSL BIOSERVICE. This allows the repeated use of the same cell culture batch in experiments. Each cell batch was routinely checked for mycoplasma infections (PCR). Thawed stock cultures were maintained in plastic culture flasks in minimal essential medium (MEM).

For purifying the cell population of pre-existing $HPRT^-$ mutants cells were exposed to HAT medium containing 100 µM hypoxanthine, 0.4 µM aminopterin, 16 µM thymidine and 10.0 µM glycine for several cell doublings (2-3 days) [9].

10.4.2. Medium

Complete Culture Medium

MEM medium supplemented with

10	%	fetal bovine serum (FBS)
100 U/100	µg/mL	penicillin/streptomycin
2	mM	L-glutamine
25	mM	HEPES
2.5	µg/mL	amphotericin B

Treatment Medium

MEM medium supplemented with

0	%	fetal bovine serum (FBS): short-term exposure
10	%	fetal bovine serum (FBS): long-term exposure
100 U/100	µg/mL	penicillin/streptomycin
2	mM	L-glutamine
25	mM	HEPES
2.5	µg/mL	amphotericin B

Selective Medium

MEM complete culture medium supplemented with thioguanine (TG, 11 µg/mL).

10.4.3. Mammalian Microsomal Fraction S9 Mix

An advantage of using *in vitro* cell cultures is the accurate control of the concentration and exposure time of cells to the test item under study. However, due to the limited capacity of cells growing *in vitro* for metabolic activation of potential mutagens, an exogenous metabolic activation system is necessary [8]. Many substances only develop mutagenic potential when they are metabolized by the mammalian organism. Metabolic activation of substances can be achieved by supplementing the cell cultures with liver microsome preparations (S9 mix).

The S9 liver microsomal fraction was prepared at BSL BIOSERVICE GmbH. Male Wistar rats were induced with Phenobarbital (80 mg/kg bw) and β-Naphthoflavone (100 mg/kg bw) for three consecutive days by oral route.

The following quality control determinations were performed:

a) Biological activity in:
- the *Salmonella typhimurium* assay using 2-aminoanthracene and benzo[*a*]pyrene
- the mouse lymphoma assay using benzo[*a*]pyrene
- the HPRT assay using 1,2-Dimethylbenz[a]anthracene.

b) Sterility Test

A stock of the supernatant containing the microsomes was frozen in ampoules of 2 and 4 mL and stored at ≤ -75°C.

The protein concentration in the S9 preparation (Lot: 280613B) was 33 mg/mL. The S9 mix preparation was performed according to Ames et al. [8].

10.4.4. S9 Mix

An appropriate quantity of the S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the concentrations below:

8 mM	$MgCl_2$
33 mM	KCl
5 mM	Glucose-6-phosphate
5 mM	NADP

in 100 mM sodium-phosphate-buffer pH 7.4. During the experiment the S9 mix was stored on ice.

10.5. Experimental Design

10.5.1. Pre-Test for Toxicity

The toxicity of the test item was determined in pre-experiments. Ten concentrations [0.010, 0.025, 0.05, 0.10, 0.25, 0.5, 1.0, 2.5, 3.5, 5 µM] were tested **with** and **without** metabolic activation. The experimental conditions in these pre-experiments were the same as described below for the main experiment with short-term exposure. For the 20 h long-term exposure assay (experiment II, only **without** metabolic activation) ten concentrations [0.25, 0.5, 1.0, 2.5, 5, 10, 25, 50, 100, 250 µM] were tested. The experimental conditions in this pre-experiment were the same as described below for the long-term exposure experiment

10.5.2. Exposure Concentrations

The test item was investigated at the following concentrations:

Experiment I

without metabolic activation:

0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5 and 10 µM

and **with** metabolic activation:

2.5, 5, 10, 25, 50, 75, 100, 200, 300 and 400 µM

Experiment II

without metabolic activation:

0.05, 0.10, 0.25, 0.50, 0.75, 1, 2, 4, 6 and 8 µM

and **with** metabolic activation:

60, 130, 160, 190, 220, 250, 280 and 310 µM

Experiment I **with** and **without** metabolic activation and experiment II **with** metabolic activation were performed as 4 h short-term exposure assay. Experiment II **without** metabolic activation was performed as a 20 h long-term exposure assay.

According to OECD Guidelines at least 8 concentrations of the test item were set up in the experiments **with** and **without** metabolic activation.

10.5.3. Experimental Performance

Seeding of the Cultures

Prior to use, cultures have been cleansed of pre-existing cells (see 10.4.1 Cells). Two or three days old exponentially growing stock cultures (more than 50% confluent) were trypsinised at 37° C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsine concentration for all subculturing steps was 0.05%.

Approximately 1.0 x 10^6 cells per concentration, solvent/negative and positive control, were seeded in complete culture medium (MEM supplemented with 10% FBS) in a culture flask, respectively (see experimental scheme).

Treatment

Approx. 24 h after seeding the cells were exposed to designated concentrations of the test item either in the presence or absence of metabolic activation in the mutation experiment. After 4 h (short time exposure) or 20 h (long time exposure) the treatment medium containing the test item was removed and the cells were washed twice with PBS. Subsequently complete medium (MEM supplemented with 10% FBS) was added. During the following expression period the cells of the logarithmic growing culture were subcultured 48 to 72 h after treatment. Additionally the cell density was measured (for toxicity criteria) and adjusted to 1 x 10^6 cells/mL.

At the end of the expression period for selection the mutants, about 4 x 10^5 cells from each treatment group, were seeded in cell culture petri dishes (diameter 90 mm) with selection medium containing 11 µg/mL thioguanine (TG) for further incubation (about one week). In experiment I at a concentration of 400 µM (**with** metabolic activation) and experiment II at a concentration of 8 µM (**without** metabolic activation) only 2 x 10^5 cells were seeded due to a very reduced number of surviving cells. At the end of the selection period, colonies were fixed and stained for counting.

The cloning efficiencies (CE) were determined in parallel to the selection of mutants. For each treatment group two 25 cm^2 flasks were seeded with approx. 200 cells to determine cloning efficiencies. After incubation for an appropriate time colonies were fixed with methanol, stained with Giemsa and counted [12].

The cloning efficiency will be calculated as follows:

$$CE\ [\%] = \frac{\text{mean of colonies (dose group)}}{200} \times 100$$

The mutation rate will be calculated as follows:

$$\text{Mutants per } 10^6 \text{ cells} = \frac{\text{mean number of mutants (dose group)}}{400\,000 \times CE[\%] / 100\ \text{(dose group)}} \times 10^6$$

10.5.4. Experimental Scheme

Subculturing of a log-phase-culture.

Day	Determination of cytotoxicity	Determination of mutation rates
1		$1.0x10^6$ cells in 30 mL medium/175 cm^2-plastic-flask for mutagenicity test, 1 flask per experimental point
2		Treatment
4 or 5	Cell count of the 175 cm^2-plastic-flask at the time point of subculturing	Subculturing of $1.0x10^6$ cells in 30 mL medium/175-cm^2-plastic-flask
7 to 10	Subculturing of 200 cells/25 cm^2-plastic-flask of the mutation experiment	Subculturing of about $4x10^5$ cells in petri dishes with selective medium; 5 petri dishes per experimental point
13 to 18	Fixation and staining	Fixation and staining

10.6. Data Recording

The data generated are recorded in the raw data. The results are presented in tables, including experimental groups with the test item, negative and positive controls. Individual colony counts for the treated and control groups are presented for both mutation induction and survival.

10.7. Acceptability of the Assay

A mutation assay is considered acceptable if it meets the following criteria:

- Negative and/or solvent controls fall within the performing laboratories historical control data range: 5-44 mutants/10^6 cells;

	-S9	+S9
Mean	24	23
Min	5	5
Max	43	44
SD	9.7	9.3
RSD [%]	40	40
n =	85	88

S9: metabolic activation
Mean: mean of mutants/10^6 cells
Min.: minimum of mutants/10^6 cells
Max.: maximum of mutants/10^6 cells
SD: standard deviation
RSD: relative standard deviation
n: number of control values

- The absolute cloning efficiency:
  ([number of positive cultures x 100] / total number of seeded cultures) of the negative and /or solvent controls is > 50%
- The positive controls (EMS and DMBA) induce significant increases (at least 3-fold increase of mutant frequencies related to the comparable negative control values and higher than the historical range of negative controls) in the mutant frequencies.

10.8. Evaluation of Results

A test is considered to be negative if there is no biological relevant increase in the number of mutants.

There are several criteria for determining a positive result [9], [12]:

- a reproducible three times higher mutation frequency than the solvent control for at least one of the concentrations;
- a concentration related increase of the mutation frequency; such an evaluation may be considered also in the case that a three-fold increase of the mutant frequency is not observed;
- if there is by chance a low spontaneous mutation rate in the corresponding negative and solvent controls a concentration related increase of the mutations within their range has to be discussed.

According to the OECD guidelines, the biological relevance of the results is the criterion for the interpretation of results, a statistical evaluation of the results is not regarded as necessary.

11. Deviations from the Study Plan

There was the following deviation from the study plan:

Concerning:

Treatment, study plan, p. 15;

Study Plan:

At the end of the expression period for selection the mutants, about 4 x 10^5 cells from each treatment group will be seeded in cell culture petri dishes (diameter 90 mm)....

Report:

At the end of the expression period for selection the mutants, about 4 x 10^5 cells from each treatment group, were seeded in cell culture petri dishes (diameter 90 mm) ... In experiment I at a concentration of 400 µM (**with** metabolic activation) and experiment II at a concentration of 8 µM (**without** metabolic activation) only 2 x 10^5 cells were seeded due to a very reduced number of surviving cells.

Reason:

For seeding out the cells for selection as described in the study plan not enough cells survived. Therefore, the number of cells seeded was reduced to 2 x 10^5 cells per petri dish. Of course this reduced number of seeded cells was considered in the calculation of the number of mutants per 10^6 cells.

This deviation did not influence the quality or integrity of the present study.

12. Results and Discussion

12.1. Results

12.1.1. Pre-Experiments

Table 1: Pre-Experiment for Toxicity, without metabolic activation

Dose Group	Concen-tration [mM]	Cell Density [cells/ml][a]	Relative Growth [%][a]
NC1	0	1140000	189.4
NC2		1050000	174.4
S1	0	629000	100
S2		575000	
1	0.010	89900	14.9
2	0.025	54300	9.0
3	0.05	24000	4.0
4	0.10	11200	1.9
5	0.25	13200	2.2
6	0.5	21400	3.6
7	1.0	14300	2.4
8	2.5	13400	2.2
9	3.5	15700	2.6
10	5.0	14600	2.4

NC: negative control / medium control
S: solvent control
a: cell density and relative growth at 1st subcultivation

Table 2: Pre-Experiment for Toxicity, with metabolic activation

Dose Group	Concen-tration [mM]	Cell Density [cells/ml][a]	Relative Growth [%][a]
NC1	0	1320000	166.0
NC2		1310000	164.8
S1	0	820000	100
S2		770000	
1	0.010	917000	115.3
2	0.025	957000	120.4
3	0.01	746000	93.8
4	0.10	506000	63.6
5	0.25	533000	67.0
6	0.5	137000	17.2
7	1.0	64500	8.1
8	2.5	15700	2.0
9	3.5	16300	2.1
10	5.0	16900	2.1

NC: negative control / medium control
S: solvent control
a: cell density and relative growth at 1st subcultivation

Table 3: Pre-Experiment for Toxicity (Experiment II), without metabolic activation

Dose Group	Concen-tration [µM]	Cell Density [cells/mL][a]	Relative Growth [%][a]
NC1	0	1550000	96.0
NC2		1610000	99.7
S1	0	1630000	100
S2		1600000	
1	0.25	1340000	83.0
2	0.5	1390000	86.1
3	1.0	1200000	74.3
4	2.5	935000	57.9
5	5	499000	30.9
6	10	241000	14.9
7	25	147000	9.1
8	50	30500	1.9
9	100	†	n.d.
10	250	†	n.d.

NC: negative control / medium control
S: solvent control
a: cell density and relative growth at 1st subcultivation
†: no viable cells
n.d.: no data

12.1.2. Experiment I, without metabolic activation

Table 4: Experiment I - Toxicity, without metabolic activation

Dose Group	Concentration [µM]	Cell Density [cells/mL][a]	Relative Growth [%][a]	Number of cells per flask			Cloning Efficiency[b] [%]
				I	II	mean	
NC1	0	1160000	108.4	168	185	177	88
NC2		1160000	108.4	183	167	175	88
S1	0	1080000	100	180	171	176	88
S2		1060000		174	171	173	86
4	0.10	1120000	104.7	164	185	175	87
5	0.25	1020000	95.3	171	185	178	89
6	0.50	1070000	100.0	164	179	172	86
7	0.75	785000	73.4	156	183	170	85
8	1.0	705000	65.9	171	184	178	89
9	2.5	665000	62.1	150	146	148	74
10	5.0	262000	24.5	153	170	162	81
11	7.5	135000	12.6	156	182	169	85
12	10	111000	10.4	157	139	148	74
EMS	300 µg/mL	1220000	114.0	176	169	173	86

NC: negative control / medium control
S: solvent control
a: cell density and relative growth at 1st subcultivation
b: mean value of cells per flask/200
EMS: Ethylmethanesulfonate [300 µg/mL]

Table 5: Experiment I – Mutagenicity, without metabolic activation

Dose Group	Concentration [µM]	Number of mutant colonies per flask[a]					Mean	SD	Mutant colonies per 10^6 cells[b]	Mutation factor
		I	II	III	IV	V				
NC1	0	3	5	6	8	13	7.0	3.41	19.83	
NC2		4	5	7	8	9	6.6	1.85	18.86	
S1	0	3	5	10	11	12	8.2	3.54	23.36	
S2		4	6	7	7	10	6.8	1.94	19.71	
4	0.10	9	11	12	12	15	11.8	1.94	33.81	1.57
5	0.25	7	9	10	13	15	10.8	2.86	30.34	1.41
6	0.50	8	12	12	17	17	13.2	3.43	38.48	1.79
7	0.75	13	13	14	15	16	14.2	1.17	41.89	1.95
8	1.0	14	14	16	16	8	13.6	2.94	38.31	1.78
9	2.5	10	14	14	16	16	14.0	2.19	47.30	2.20
10	5.0	7	8	9	10	12	9.2	1.72	28.48	1.32
11	7.5	12	14	16	18	18	15.6	2.33	46.15	2.14
12	10	5	10	10	12	15	10.4	3.26	35.14	1.63
EMS	300 µg/mL	67	75	78	59	76	71.0	7.07	205.80	9.56

NC: negative control / medium control
S: solvent control
a: number of mutant colonies in flask I to V
b: mean mutant colonies x 10^6 / (400000 x Cloning Efficiency/100)
EMS: Ethylmethanesulfonate [300 µg/mL]

12.1.3. Experiment I, with metabolic activation

Table 6: Experiment I - Toxicity, with metabolic activation

Dose Group	Concen-tration [mM]	Cell Density [cells/mL][a]	Relative Growth [%][a]	Number of cells per flask			Cloning Efficiency[b] [%]
				I	II	mean	
NC1	0	1510000	118.0	155	163	159	80
NC2		1440000	112.5	130	146	138	69
S1	0	1460000	100	146	148	147	74
S2		1100000		123	144	134	67
2	2.5	1040000	81.3	146	153	150	75
3	5	1310000	102.3	145	156	151	75
4	10	1440000	112.5	155	176	166	83
5	25	1260000	98.4	151	157	154	77
6	50	1010000	78.9	123	157	140	70
7	75	1160000	90.6	110	148	129	65
8	100	1250000	97.7	139	156	148	74
9	200	842000	65.8	116	129	123	61
10	300	253000	19.8	72	92	82	41
11	400	146000	11.4	416	428	422	21
DMBA	0.8 µg/mL	1480000	115.6	140	143	142	71
DMBA	1.0 µg/mL	1180000	92.2	147	165	156	78

NC: negative control / medium control
S: solvent control
a: cell density and relative growth at 1st subcultivation
b: mean value of cells per flask/200
DMBA : 7,12-Dimethylbenz(*a*)anthracene [µg/mL]

Table 7: Experiment I – Mutagenicity, with metabolic activation

Dose Group	Concen-tration [mM]	Number of mutant colonies per flask [a]					Mean	SD	Mutant colonies per 10^6 cells[b]	Mutation factor
		I	II	III	IV	V				
NC1	0	8	15	12	10	12	11.4	2.33	35.85	
NC2		10	13	14	13	8	11.6	2.24	42.03	
S1	0	8	7	6	9	8	7.6	1.02	25.85	
S2		3	3	5	7	11	5.8	2.99	21.72	
2	2.5	4	6	3	2	0	3.0	2.00	10.03	0.42
3	5	5	4	6	4	4	4.6	0.80	15.28	0.64
4	10	3	10	6	9	10	7.6	2.73	22.96	0.97
5	25	2	8	4	8	7	5.8	2.40	18.83	0.79
6	50	18	10	11	19	10	13.6	4.03	48.57	2.04
7	75	7	6	6	13	7	7.8	2.64	30.23	1.27
8	100	8	10	19	10	12	11.8	3.82	40.00	1.68
9	200	17	16	18	12	17	16.0	2.10	65.31	2.75
10	300	16	18	11	10	15	14.0	3.03	85.37	3.59
11	400	15	15	8	18	9	13.0	3.85	308.06	12.95
DMBA	0.8 µg/mL	59	72	69	100	81	76.2	13.82	269.26	11.32
DMBA	1.0 µg/mL	115	108	96	79	107	101.0	12.57	323.72	13.61

NC: negative control / medium control
S: solvent control
a: number of mutant colonies in flask I to V
b: mean mutant colonies x 10^6 / (400000 x Cloning Efficiency/100)
DMBA: 7,12-Dimethylbenz(*a*)anthracene [µg/mL]

12.1.4. Experiment II, without metabolic activation

Table 8: Experiment II - Toxicity, without metabolic activation

Dose Group	Concentration [µM]	Cell Density [cells/mL][a]	Relative Growth [%][a]	Number of cells per flask			Cloning Efficiency[b] [%]
				I	II	mean	
NC1	0	2180000	107.4	175	80	180	90
NC2		2020000	99.5	139	69	140	70
S1	0	2020000	100	162	74	168	84
S2		2040000		155	67	167	84
3	0.05	2070000	102.0	165	75	168	84
4	0.10	2170000	106.9	145	75	157	78
5	0.25	1920000	94.6	158	83	161	80
6	0.50	2010000	99.0	128	77	135	68
7	0.75	1960000	96.6	149	70	157	79
8	1.0	1910000	94.1	155	65	157	79
9	2.0	1730000	85.2	117	74	135	68
10	4.0	1030000	50.7	92	61	109	54
11	6.0	554000	27.3	65	41	73	36
12	8.0	378000	18.6	43	21	46	23
EMS	300 µg/mL	1650000	81.3	131	71	133	66

NC: negative control / medium control
S: solvent control
a: cell density and relative growth at 1st subcultivation
b: mean value of cells per flask/200
EMS: Ethylmethanesulfonate [300 µg/mL]

Table 9: Experiment II – Mutagenicity, without metabolic activation

Dose Group	Concentration [µM]	Number of mutant colonies per flask [a]					Mean	SD	Mutant colonies per 10^6 cells[b]	Mutation factor
		I	II	III	IV	V				
NC1	0	4	8	9	10	10	8.2	2.23	22.84	
NC2		6	7	9	11	11	8.8	2.04	31.54	
S1	0	7	9	10	10	12	9.6	1.62	28.57	
S2		5	7	8	11	14	9.0	3.16	26.95	
3	0.05	2	4	5	6	9	5.2	2.32	15.48	0.56
4	0.10	6	11	11	12	14	10.8	2.64	34.50	1.24
5	0.25	2	9	10	13	16	10.0	4.69	31.15	1.12
6	0.50	3	3	5	11	11	6.6	3.67	24.44	0.88
7	0.75	7	8	9	10	12	9.2	1.72	29.30	1.06
8	1.0	5	6	7	8	9	7.0	1.41	22.29	0.80
9	2.0	14	17	19	19	22	18.2	2.64	67.41	2.43
10	4.0	25	28	29	34	35	30.2	3.76	139.17	5.01
11	6.0	10	12	11	13	18	12.8	2.79	88.28	3.18
12	8.0	1	2	2	3	6	2.8	1.72	60.87	2.19
EMS	300 µg/mL	165	177	167	184	172	173.0	6.90	652.83	23.52

NC: negative control / medium control
S: solvent control
a: number of mutant colonies in flask I to V
b: mean mutant colonies x 10^6 / (400000 x Cloning Efficiency/100)
EMS: Ethylmethanesulfonate [300 µg/mL]

12.1.5. Experiment II, with metabolic activation

Table 10: Experiment II - Toxicity, with metabolic activation

Dose Group	Concentration [µM]	Cell Density [cells/ml][a]	Relative Growth [%][a]	Number of cells per flask			Cloning Efficiency[b] [%]
				I	II	mean	
NC1	0	1390000	100.0	219	203	211	106
NC2		1340000	96.4	179	205	192	96
S1	0	1310000	100	200	195	198	99
S2		1470000		186	199	193	96
2	60	1330000	95.7	184	199	192	96
4	130	1090000	78.4	170	158	164	82
5	160	851000	61.2	150	149	150	75
6	190	621000	44.7	135	126	131	65
7	220	768000	55.3	153	142	148	74
8	250	555000	39.9	121	113	117	59
9	280	368000	26.5	106	108	107	54
10	310	252000	18.1	84	78	81	41
DMBA	0.8 µg/mL	1410000	101.4	174	168	171	86
DMBA	1.0 µg/mL	1350000	97.1	165	155	160	80

NC: negative control / medium control
S: solvent control
a: cell density and relative growth at 1st subcultivation
b: mean value of cells per flask/200
DMBA: 7,12-Dimethylbenz(*a*)anthracene [µg/mL]

Table 11: Experiment II – Mutagenicity, with metabolic activation

Dose Group	Concentration [µM]	Number of mutant colonies per flask[a]					Mean	SD	Mutant colonies per 10^6 cells[b]	Mutation factor
		I	II	III	IV	V				
NC1	0	7	13	14	17	17	13.6	3.67	32.23	
NC2		4	9	10	11	12	9.2	2.79	23.96	
S1	0	3	3	6	8	9	5.8	2.48	14.68	
S2		6	8	9	10	10	8.6	1.50	22.34	
2	60	9	12	13	13	13	12.0	1.55	31.33	1.69
4	130	11	14	16	18	22	16.2	3.71	49.39	2.67
5	160	8	11	15	16	16	13.2	3.19	44.15	2.38
6	190	8	9	9	9	12	9.4	1.36	36.02	1.95
7	220	8	11	13	15	15	12.4	2.65	42.03	2.27
8	250	12	19	19	19	20	17.8	2.93	76.07	4.11
9	280	26	28	29	27	31	28.2	1.72	131.78	7.12
10	310	12	12	13	18	19	14.8	3.06	91.36	4.94
DMBA	0.8 µg/mL	61	47	50	55	45	51.6	5.78	150.88	8.15
DMBA	1.0 µg/mL	93	120	93	86	102	98.8	11.75	308.75	16.68

NC: negative control / medium control
S: solvent control
a: number of mutant colonies in flask I to V
b: mean mutant colonies x 10^6 / (400000 x Cloning Efficiency/100)
DMBA: 7,12-Dimethylbenz(*a*)anthracene [µg/mL]

12.2. Discussion

The test item Araldite PT 910 (TK 30041) was assessed for its potential to induce gene mutations at the HPRT locus using V79 cells of the Chinese hamster.

The main experiments were carried out **without** and **with** metabolic activation. The experiments **with** metabolic activation were performed by including liver microsomes and NADP for efficient detection of a wide variety of carcinogens requiring metabolic activation.

The selection of the concentrations used in the main experiments was based on data from the pre-experiments according to the OECD guideline 476.

In experiment I 10 µM (**without** metabolic activation) and 400 µM (**with** metabolic activation) were selected as the highest concentrations. In experiment II 8 µM (**without** metabolic activation) and 310 µM (**with** metabolic activation) were selected as the highest concentrations. Experiment I **with** and **without** metabolic activation and experiment II **with** metabolic activation were performed as a 4 h short-term exposure assay. Experiment II was performed as 20 h long time exposure assay (**without** metabolic activation).

The pH-value detected with the test item was within the physiological range.

The test item was investigated at the following concentrations:

Experiment I

without metabolic activation:

0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5 and 10 µM

and **with** metabolic activation:

2.5, 5, 10, 25, 50, 75, 100, 200, 300 and 400 µM

Experiment II

without metabolic activation:

0.05, 0.10, 0.25, 0.50, 0.75, 1, 2, 4, 6 and 8 µM

and **with** metabolic activation:

60, 130, 160, 190, 220, 250, 280 and 310 µM

Precipitation:

No precipitation of the test item was noted in any of the experiments.

Toxicity:

A biologically relevant growth inhibition (reduction of relative growth below 70%) was observed after the treatment with the test item in experiment I and II **with** and **without** metabolic activation.

In experiment I **without** metabolic activation the relative growth was 10.4% for the highest concentration (10 µM) evaluated. The highest biologically relevant concentration evaluated **with** metabolic activation was 400 µM with a relative growth of 11.4%.

In experiment II **without** metabolic activation the relative growth was 18.6% for the highest concentration (8 µM) evaluated. The highest concentration evaluated **with** metabolic activation was 310 µM with a relative growth of 18.1%.

Mutagenicity:

In experiment I **without** metabolic activation mutant values of the negative controls, the solvent controls and most mutant values of test item concentrations found were within the historical control data of the test facility BSL BIOSERVICE (about 5-43 mutants per 10^6 cells). No dose-response relationship could be observed. The mutation frequencies found in the groups treated with the test item did not show a biologically relevant increase as compared to the solvent controls.

Mutation frequencies with the negative control were found to be 19.83 and 18.86, of the solvent control 23.36 and 19.71 mutants/10^6 cells and in the range of 28.48 to 47.30 mutants/10^6 cells with the test item, respectively. The highest mutation rate (compared to the solvent control values) of 2.20 was found at a concentration of 2.5 µM with a relative growth of 62.1%.

With metabolic activation the mutant values of the negative controls and the solvent controls found were within the historical control data of the test facility BSL BIOSERVICE (about 5-44 mutants per 10^6 cells).

Mutation frequencies with the negative control were found to be 35.85 and 42.03, of the solvent control 25.85 and 21.72 mutants/10^6 cells and in the range of 10.03 to 308.06 mutants/10^6 cells with the test item, respectively.

The mutation frequencies found in the groups treated with the test item up to concentrations of 100 µM did not show a biologically relevant increase as compared to the solvent controls. At concentrations of 200 µM (65.31 mutants per 10^6 cells) 300 µM (85.37 mutants per 10^6 cells) and 400 µM (308.06 mutants per 10^6 cells) the mutant values found were above the historical control data range. At a concentration of 200 µM a mutation rate (compared to the solvent control values) of 2.75 was found with a relative growth of 65.8%. For concentrations of 300 µM and 400 µM a mutation rate (compared to the solvent control values) of 3.59 and 12.95, respectively, were found with a relative growth of 19.8% and 11.4%, respectively. Thus, at concentrations of 300 µM and 400 µM the threshold value of 3.0 as well as the range of the historical control data were exceeded. Moreover, a dose-response relationship was observed.

In experiment II **without** metabolic activation the mutant values of the negative controls and the solvent controls and test item concentrations found were within the historical control data of the test facility BSL BIOSERVICE (about 5-43 mutants per 10^6 cells).

Mutation frequencies with the negative control were found to be 22.84 and 31.54, of the solvent control 28.57 and 26.95 mutants/10^6 cells and in the range of 15.48 to 139.17 mutants/10^6 cells with the test item, respectively.

The mutation frequencies found in the groups treated with the test item up to concentrations of 1 µM did not show a biologically relevant increase as compared to the solvent controls. At concentrations of 2 µM (67.41 mutants per 10^6 cells), 4 µM (139.17 mutants per 10^6 cells), 6 µM (88.28 mutants per 10^6 cells) and 8 µM (60.87 mutants per 10^6 cells) the mutant values found were above the historical control data range. For concentrations of 2 µM and 8 µM a mutation rate (compared to the solvent control values) of 2.43 and 2.19, respectively, was found with a relative growth of 85.2% and 18.6%, respectively. For concentrations of 4 µM and 6 µM a mutation rate (compared to the solvent control values) of 5.01 and 3.18, respectively, was found with a relative growth of 50.7% and 27.3%, respectively. Thus, at concentrations of 4 µM and 6 µM the threshold value of 3.0 as well as the range of the historical control data were exceeded.

With metabolic activation the mutant values of the negative controls and the solvent controls found were within the historical control data of the test facility BSL BIOSERVICE (about 5-44 mutants per 10^6 cells).

Mutation frequencies with the negative control were found to be 32.23 and 23.96, of the solvent control 14.68 and 22.34 mutants/10^6 cells and in the range of 31.33 to 131.78 mutants/10^6 cells with the test item, respectively.

The mutation frequencies found in the groups treated with the test item up to concentrations of 220 µM did not show a biologically relevant increase as compared to the solvent controls. At concentrations of 250 µM (76.07 mutants per 10^6 cells), 280 µM (131.78 mutants per 10^6 cells) and 310 µM (91.36 mutants per 10^6 cells) the mutant values found were above the historical control data range. The corresponding mutation rates (compared to the solvent control values) found were 4.11 (250 µM), 7.12 (280 µM) and 4.94 (310 µM), with a relative growth of 39.9%, 26.5% and 18.1%, respectively. Thus, at concentrations of 250 µM, 280 µM and 310 µM the threshold value of 3.0 as well as the range of the historical control data were exceeded.

DMBA (0.8 and 1.0 µg/mL) and EMS (300 µg/mL) were used as positive controls and showed distinct and biologically relevant effects in mutation frequency.

13. Conclusion

In conclusion, in the described *in vitro* cell gene mutagenicity test under the experimental conditions reported, the test item Araldite PT 910 (TK 30041) is considered to be mutagenic in the HPRT locus using V79 cells of the Chinese Hamster.

14. Distribution of the Report

1 original (paper):	Sponsor
1 copy (paper):	BSL BIOSERVICE
1 copy (electronic):	Sponsor

15. References

15.1. Guidelines

[1] Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on August 28, 2013 (BGBl. I S. 3498)

[2] Konsens-Dokument der Bund-Länder-Arbeitsgruppe Gute Laborpraxis ("Consensus Document of the National and Länder Working Party on Good Laboratory Practice") on the archiving and storage of records and materials, 5 May 1998

[3] OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998

[4] Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 476, "*In vitro* Mammalian Cell Gene Mutation Tests" adopted July 21, 1997

[5] Commission Regulation (EC) No 440/2008, L142, Annex Part B, B 17, "*In vitro* Mammalian Cell Gene Mutation Test", dated May 30, 2008

[6] EPA Health Effects Test Guidelines, OPPTS 870.5300 "*In vitro* Mammalian Cell Gene Mutation Test" EPA 712-C-98-221, August 1998

15.2. Literature

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[13] D.J. Kirkland; M. Hayashi; D. Jacobson-Kram; P. Kasper; J.T. MacGregor; L. Müller; Y. Uno (2007). The International Workshops on Genotoxicity Testing (IWGT): History and achievements. Mutat. Res. 627, 1-4

15.3. Internal BSL BIOSERVICE SOPs

Standard Operating Procedures (SOPs), No. 4-6-5, No. 15-1-1, No. 15-1-2, No.15-2-5

16. Appendix

16.1. Appendix 1: Historical Laboratory Control Data

Table 12: Historical Laboratory Control Data

	NC		PC	
	-S9	+S9	EMS	DMBA
Mean	24	23	400	483
Min	5	5	123	140
Max	43	44	907	866
SD	9.7	9.3	203	197
RSD [%]	40	40	51	41
n =	85	88	82	68

NC: negative control
PC: positive controls (-S9 EMS; +S9 DMBA)
S9: metabolic activation
Mean: mean of mutants/10^6 cells
Min.: minimum of mutants/10^6 cells
Max.: maximum of mutants/10^6 cells
SD: standard deviation
RSD: relative standard deviation
n: number of control values

16.2. Appendix 2: Certificate of Analysis

Huntsman Advanced
Materials(Switzerland)GmbH
Plant Monthey
Analytical Quality Control
1870 Monthey
Switzerland

Dr. Benoît Zufferey, Quality Manager
benoit_zufferey@huntsman.com

HUNTSMAN
Enriching lives through innovation

Page
1 of 1

CERTIFICATE OF ANALYSIS

according to ISO 10474 3.1B

Our Reference :
Phone No. :
FAX No. :
E-Mail :

Print Date : 23.10.2013

Product Designation: ARALDITE PT 910 20KG 87

Product Number	: 152607500	Your Product Number	:
Customer No	:	Our Vendor Number	:
Order No	:	Your Order Number	:
Delivery No.	:	Delivery Date	:
Inspection Date	: 03.06.2013	Batch Number	: AAC0647400
		Expiry date	: 17.05.2015

Characteristic	Method	Results	Unit	Min	Max
Appearance		OK			
Epoxide index	ISO 3001:1999	6,86	Eq/kg	6,50	7,10
Total Chlorine	AMTM 116:2002	0,60	%	0,00	1,00
Ionic Chlorine	AMTM 116:2002	1	ppm	0	20
Melting temperature, DSC	ISO 11357-2:1999	94	°C	90	102

It is hereby certified, that the material indicated above has been inspected and tested in accordance with the Huntsman Quality Assurance system. It conforms in all respects to the specification relevant thereto.

The test results given above were carried out on samples taken after completion of the manufacturing process and during discharge into the product containers.

This document has been generated automatically from the data of our quality database and does not require a signature.

Huntsman Advanced Materials (Europe) BVBA
Everslaan 45
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Belgium